PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BLOcp263/79P	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IB 03/03397	International filing date *(day/month/year)* 11.07.2003	Priority date *(day/month/year)* 11.07.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/315		
Applicant COMMISSARIAT A L'ENERGIE ATOMIQUE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27.01.2004	27.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Marinoni, J-C Telephone No. +49 89 2399-8563

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/03397

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-27 as originally filed

Sequence listings part of the description, Pages

1-4 as originally filed

Claims, Numbers

1-23 filed with telefax on 29.11.2004

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-23
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	1-8,11,15-22
	No:	Claims	9,10,12-14,23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	none

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application relates to a protein derived of *S. pneumoniae* PBP2x and consisting of 4 specific domains of *S. pneumoniae* PBP2x separated by 1 to 7 amino acids and the nucleic acid molecules encoding them, as well as vectors comprising said nucleic acid molecule, cells transformed with said vector. The application also relates to uses of the protein of claims 1-7 for screening antibiotics, to methods for identifying antibiotics by using a crystal of the protein of claims 1-7. The application also relates to peptides of PBP2x, antibodies, and primers.

2. Reference is made to the following documents:

D1: GORDON E ET AL: "The crystal structure of the penicillin-binding protein 2x from Streptococcus pneumoniae and its acyl-enzyme form: Implication in drug resistance." JOURNAL OF MOLECULAR BIOLOGY, vol. 299, no. 2, 2000, pages 477-485

D2: WO 98/48041 A (MAX PLANCK GESELLSCHAFT ;HAKENBECK REGINE (DE)) 29 October 1998

D3: LAIBLE ET AL: "Nucleotide sequences of the pbpX genes encoding the penicillin-binding proteins 2x from Streptococcus pneumoniae R6 and a cefotaxime-resistant mutant, C506" MOLECULAR MICROBIOLOGY, vol. 3, no. 10, 1989, pages 1337-1348

D4: PARES S ET AL.: "X-ray structure of Streptococcus pneumoniae PBP2x, a primary penicillin target enzyme" NATURE STRUCTURAL BIOLOGY, vol. 3, no. 3, March 1996 (1996-03), pages 284-289, XP009010741

D5: DESSEN ANDREA ET AL: "Crystal structure of PBP2x from a highly penicillin-resistant Streptococcus pneumoniae clinical isolate. A mosaic framework containing 83 mutations." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 48, 30 November 2001, pages 45106-45112

D6: MOUZ NICOLAS ET AL: "Mutations in the active site of penicillin-binding protein PBP2x from Streptococcus pneumoniae: Role in the specificity for beta-lactam antibiotics." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 27, 2 July 1999, pages 19175-19180

D7: MOUZ N ET AL: "Identification of a structural determinant for resistance to beta-lactam antibiotics in Gram-positive bacteria." PROC. NATL. ACAD. SCI. USA, vol. 95, no. 23, 10 November 1998, pages 13403-13406

D8: ASAHI Y ET AL: "Diversity of substitutions within or adjacent to conserved amino acid motifs of penicillin-binding protein 2X in cephalosporin-resistant Streptococcus pneumoniae isolates." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 43, no. 5, May 1999, pages 1252-1255

3. None of the available documents discloses any of the subject-matter of **claims 1-22**. **Claims 1-22** therefore meet the requirements of Art. 33(2) PCT concerning novelty.

4. The technical problem to be solved in the present application resides in obtaining "PBP2x [proteins] which are more crystallizable in a reproducible manner and which have a better diffraction power" (see page 4, lines 24-26).
The solution to this technical problem consists in the provision of the polypeptide as defined in **claim 1**.

5. **D1** explicitely mentions that regions aa49-70, aa93-182 and aa232-aa253 of the PBP2x proteins could not be resolved due to their instability ("severely disordered N terminal domain"). No biological function has been reported for the N-terminal domain (see **D4**, page 284, right-hand column, lines 31-32), whereas the transpeptidase domain comprise the active site at position 337 is situated between positions 266 and 616. The function of the C terminal domain (aa635-750) is unknown but it is stable and its structure is resolved (see **D4** and **D1**). Moreover, the data analysis in **D1** starts with amino acid 71, since (i) region 49-71 could not be resolved due to its instability and (ii) region 1-48 corresponds to the cytoplasmic region and transmembrane helix which prevent solubilization of the recombinant protein (see **D5**, "Experimental procedures").
Therefore the skilled person would want to obtain a similar PBP2x protein wherein these domains have been deleted to stabilise the protein and hence obtain crystals more easily/efficiently and/or in larger amounts in order to carry out further analysis.
Despite the unclarity of the sequence claimed due to the fact that the 1-7 amino acids preceding each of the specific regions referred to in claim 1 are left undefined, it is considered that the skilled person would not necessarily omit from his/her construct the amino acids located between position 200 and 218 and would therefore not obtain the claimed proteins.
The subject-matter of **claims 1-8, 11, 15-21 and 22** insofar as it relates to the proteins of **claims 1-7** therefore meets the requirements of Art. 33(3) PCT

concerning inventive step.

6. The peptides of **claim 9**, the antibodies of **claim 10**, the primers and probes of **claim 12-14** do not solve the technical problem underlying the present application. Since they are left undefined, the primers of **claim 13** are merely seen as variants of obvious primers of PBP2x that the skilled person would use in view of **D1-D7**. The subject-matter of said claims therefore does not meet the requirements of Art. 33(3) PCT.
Consequently, the subject-matter of the newly introduced **claim 23** does not meet said requirements of Art. 33(3) PCT either.

CLAIMS

1. Protein derived from a *Streptococcus pneumoniae* PBP2x, characterized in that it consists of a concatenation of the fragments corresponding respectively to the amino acids located between positions 74 to 90, 186 to 199, 218 to 228 and 257-750, with reference to the sequence of the PBP2x protein of the strain R6 (SWISSPROT P14677 or GENBANK 18266817), each one of said fragments being preceded by a peptide fragment of 1 to 7 amino acids.

2. Protein according to Claim 1, characterized in that said peptide fragment comprises amino acids of said *Streptococcus pneumoniae* PBP2x protein located between positions -1 to -7, relative to the residues at positions 74, 186, 218 and 257, and/or between positions +1 to +7, relative to the residues at positions 90, 199 and 228, as defined in Claim 1.

3. Protein according to Claim 1 or Claim 2, characterized in that said peptide fragment comprises amino acids chosen from alanine (A), serine (S), glycine (G) and threonine (T).

4. Protein according to any one of Claims 1 to 3, characterized in that it is derived from a β-lactam-resistant strain of *S. pneumoniae*.

5.Protein according to any one of Claims 1 to 3, characterized in that it has the sequence SEQ ID No. 1.

6.Protein according to any one of Claims 1 to 5, characterized in that it comprises a substitution of at least one methionine residue with a selenomethionine residue.

7.Protein according to any one of Claims 1 to 6, characterized in that it is associated with a ligand.

8.Protein according to any one of Claims 1 to 7, characterized in that it is in the form of a crystal.

9.Peptide, characterized in that it consists of a fragment of at least 7 amino acids of the mini-PBP2x protein, according to any one of Claims 1 to 6, which peptide includes at least one residue chosen from those located at positions 74, 90, 186, 199, 218, 228 and 257 as defined in Claim 1.

10.Antibodies, characterized in that they are directed against a peptide according to Claim 9.

11.Isolated nucleic acid molecule, characterized in that it is selected from the group consisting of the sequences encoding a mini-PBP2x according to any one of Claims 1 to 6 and the sequences complementary to the preceding sequences, which are sense or antisense.

12.Pair of primers, characterized in that it has the sequence SEQ ID Nos. 2-3.

13. Primers, characterized in that they comprise a sequence of approximately 10 to 30 nucleotides corresponding to that located at the junction of the peptide fragments of 1 to 7 amino acids and the fragments of PBP2x as defined in Claim 1.

14.Primers according to Claim 13, characterized in that they have a sequence selected from the group consisting of the sequences SEQ ID Nos. 4 to 9.

15.Recombinant vector, characterized in that it comprises an insert selected from the group consisting of the nucleic acid molecules encoding a mini-PBP2x according to Claim 11.

16.Expression vector according to Claim 15, characterized in that it consists of a prokaryotic vector.

17.Cells transformed with a recombinant vector according to either one of Claims 15 and 16.

18.Cells according to Claim 17, characterized in that they are prokaryotic cells.

19.Use of a mini-PBP2x according to any one of Claims 1 to 8, for screening antibiotics.

20.Method for screening antibiotics, characterized in that it comprises at least the following steps:

a_1)bringing a mini-PBP2x according to any one of Claims 1 to 7 into contact with a test substance,

b_1)detecting, by any suitable means, the binding of said test molecule with the mini-PBP2x and/or the inhibition of the activity of said mini-PBP2x resulting from this binding, and

c_1)selecting and identifying the active substances capable of binding to the mini-PBP2x and/or of inhibiting the activity of said mini-PBP2x, which can be used as antibiotics.

21.Method for identifying antibiotics, characterized in that it comprises at least the following steps:

a_2)preparing crystals from a mini-PBP2x according to any one of Claims 1 to 7,

b_2)determining the three-dimensional structure of said mini-PBP2x from the crystal obtained in a_2), and

c_2)identifying active substances capable of binding to the mini-PBP2x and/or of inhibiting the activity of said mini-PBP2x, which can be used as antibiotics.

22. Screening kit for implementing the method according to Claim 20 or Claim 21, characterized in that it includes at least one protein, according to any one of Claims 1 to 8.

23. Screening kit for implementing the method according to Claim 20 or Claim 21, characterized in that it further includes at least one antibody according to claim 10.